Relationship between in vitro growth inhibition of pathogens and suppression of preemergence damping-off and postemergence root rot of white bean seedlings in the greenhouse by bacteria

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One hundred and twenty diverse bacterial strains were screened under greenhouse conditions for their ability to protect white bean seedlings from preemergence damping-off caused by *Pythium ultimum* and *Rhizoctonia solani* and postemergence root rot by *Fusarium solani* f.sp. *phaseoli*. Preemergence mortality or root rot increased with an increase in the inoculum concentration of fungal isolates. For further testing, 200 propagules/g soil of *P. ultimum*, 3 propagules/g soil of *R. solani*, and log 3 conidia/g vermiculite of *F. solani* f.sp. *phaseoli* were used, as these rates provided an optimal level (approximately 50%) of disease severity. Bacterial strains suspended in sterile distilled water were added to pathogen-amended soil or vermiculite at log 7 colony-forming units/g soil or vermiculite prior to seeding. Final healthy stand and root rot were recorded 4 weeks after planting. Nine bacterial strains on *P. ultimum*, five on *R. solani*, and nine on *F. solani* f.sp. *phaseoli* provided significant ($P = 0.05$) suppression of disease severity compared with the nonbacterized control. Bacterial strains were also tested in vitro against the mycelial growth of the fungi on solid and liquid media. There was no relationship between the ability of bacterial strains to inhibit fungal vegetative growth on solid culture media and their ability to suppress pathogen activity in the greenhouse, but, for a few strains, the reduction in disease was linked to reduced growth of the pathogens in liquid media.

Key words: White bean, damping-off, root rot, *Pythium ultimum*, *Rhizoctonia solani*, *Fusarium solani* f.sp. *phaseoli*.

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Cent vingt souches bactériennes différentes ont été soumises à une étude en serre pour vérifier leur aptitude à protéger les plantules de haricot blanc, lors de la préémergence, contre la fonte des semis causée par le *Pythium ultimum* et le *Rhizoctonia solani* et, lors de la postémergence, contre la pourriture racinaire causée par le *Fusarium solani* f.sp. *phaseoli*. La mortalité en préémergence et la pourriture racinaire ont augmenté avec l'acroissement des concentrations des inoculums des isolats fongiques. Deux cents propagules par gramme de sol de *P. ultimum*, 3 propagules/g de sol de *R. solani* et log 3 conidies/g de vermiculite de *F. solani* f.sp. *phaseoli* ont été utilisées comme base d'essais ultérieurs, vu que ces taux procuraient un niveau optimum de sévérité de la maladie, soit environ 50%. Les souches bactériennes, suspendues dans l'eau distillée stérile, ont été ajoutées à du sol ou de la vermiculite amendés en pathogènes à raison de log 7 unités formatrices de colonies/g de sol ou de vermiculite, avant l'ensemencement. Quatre semaines après le repiquage, la pourriture racinaire et l'état de santé final des plantules ont fait l'objet d'un relevé. Neuf souches bactériennes sur le *P. ultimum*, cinq sur le *R. solani* et neuf sur le *F. solani* f.sp. *phaseoli* ont causé une suppression significative ($P = 0,05$) de la sévérité de la maladie, par comparaison au témoin. Les souches bactériennes ont aussi été testées in vitro contre la croissance mycélienne des champignons sur des milieux de culture solides et dans des milieux liquides. Aucune relation n'a été obtenue entre la capacité des souches bactériennes d'inhiber la croissance végétative fongique sur les milieux solides, ainsi que leur aptitude à supprimer l'activité des organismes pathogènes en serre mais, pour quelques souches, la réduction de la maladie a été liée à une réduction de la croissance des pathogènes dans les milieux liquides.

Mots clés : haricot blanc, fonte des semis, pourriture racinaire, *Pythium ultimum*, *Rhyzoctonia solani*, *Fusarium solani* f.sp. *phaseoli*.

[Traduit par la rédaction]

Introduction

Preemergence damping-off caused by *Pythium ultimum* Trow and *Rhizoctonia solani* Kuehn and postemergence root rot caused by *Fusarium solani* (Mart.) Appel and Wr. f.sp. *phaseoli* (Burk.) Snyd. and Hans. are serious diseases of white bean (*Phaseolus vulgaris* L.) (Burke and Kraft 1974; Hall 1983). Disease losses are particularly severe where beans are cropped in consecutive years (Buonassisi et al. 1986), owing to concomitant increases in pathogen populations (Lumsden et al. 1976). Yield losses ranging from 35 to 80% of crop potential are not unusual. Crop rotation does not provide sufficient control. Disease-tolerant cultivars are beneficial (Burke and Kraft 1974), but for other reasons, growers prefer cultivars with no resistance. Furthermore, introduction of tolerant bean cultivars may bring only short-term success, as selection pressure for strains of the pathogens that overcome resistance genes is expected to occur. Some fungicides control the disease; however, inconsistency of protection is frequently encountered (Brent 1987).

Soil bacteria including species of *Azotobacter*, *Bacillus*, *Clostridium*, *Serratia*, and *Pseudomonas*, and coryneform-like

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species have been shown to reduce soil-borne fungal diseases when applied as seed, soil, or root inoculants (Cook and Baker 1983). In addition, rhizobacteria can enhance the growth and yield of many crop plants even in the absence of plant pathogens (Howell and Stipanovic 1980; Kloepper et al. 1986, 1988; Reddy and Rahe 1989; Reddy and Patrick 1992; Weller 1988). A number of commercial products in which bacterial agents are the active ingredients are now in commercial production. For example, *Bacillus subtilis* (Quantum-4000™) is sold as a rhizobacterial inoculant for peanuts (Gustafson Inc., Dallas, Tex.).

Previous work showed that several bacterial strains isolated from diverse locations could promote growth of several agricultural crops and suppress some soil-borne pathogens (Kloepper et al. 1986, 1988; Reddy et al. 1990, 1991; Young et al. 1991). In the present study we report the results of screening of 120 bacterial strains of diverse origins for their ability to suppress damping-off diseases of white bean caused by *Pythium ultimum*, *R. solani*, and of postemergence root rot caused by *F. solani* f.sp. *phaseoli* under greenhouse conditions. Also, bioassays were designed to indicate mechanisms involved in biological control. A preliminary report of this study was published (Reddy et al. 1990).

Methods and materials

Bacterial cultures and inoculum preparation

The bacteria used in this study were isolates from diverse soils and root regions (Kloepper et al. 1986, 1988). Strains were identified by determining their growth on various media, their ability to form pigments, and their responses to biochemical tests included in API-20 E strips (API Laboratory Products Ltd., St. Laurent, Que.). The selected strains possessed one or several of the following characteristics: (*i*) in vitro antibiosis against various fungal pathogens, (*ii*) promotion of rhizobial root nodulation, (*iii*) enhancement of root and shoot growth of various crops and vegetables, and (*iv*) ability to produce plant growth regulators (Kloepper et al. 1986, 1988; Reddy et al. 1990, 1991; Young et al. 1991; M.S. Reddy, R.K. Hynes, and G. Lazarovits, unpublished results). Purified bacterial strains were grown for 48 h at 25°C in 25 mL sterile tryptic soy broth (TSB) (Difco, Detroit, Mich.) on a reciprocating shaker (80 rpm). Bacteria were pelleted by centrifugation for 20 min at 10 000 × *g*. Bacterial cells were then washed (twice) in 0.1 M phosphate buffer (PB) (pH 6.8), resuspended in TSB amended with 20% sterile glycerol, and frozen in vials at −80°C. In each experiment a new vial of bacteria was used. The bacteria were streaked onto pseudomonas agar F (PAF) (Difco) and checked for purity after incubation for 24 h at 30°C.

Bacterial inoculum was prepared on PAF plates at 30°C. After 48 h the bacterial lawns were scraped into 0.1 M PB (10 mL/plate). The suspension was centrifuged for 15 min at 10 000 × *g*. The pellet was rinsed with PB, resuspended in sterile distilled water (SDW), and adjusted to a concentration of log 9–10 colony-forming units (cfu)/mL. The cell concentrations were determined by measuring the absorbence of the suspension (A = 520 nm). Correlations between the absorbence and the number of living cells were established earlier by the plate count procedure.

Fungal isolates and inoculum preparation

Fungal isolates were obtained from Dr. R. Baker, Department of Botany and Plant Pathology, Colorado University, Fort Collins (*Pythium ultimum* isolate 67-1), from Ciba-Geigy, Switzerland (*R. solani* CG160), and from Dr. R. Hall, Department of Environmental Biology, University of Guelph, Guelph, Ont. (*F. solani* f.sp. *phaseoli*). *Pythium ultimum* was maintained on hemp seed in sterile water or 2% water agar (WA), while *R. solani* and *F. solani* f.sp. *phaseoli* were maintained in autoclaved soil at 25°C.

Inoculum of *Pythium ultimum* was produced by growing the fungus for 2 weeks at 20°C on rolled oat and WA (Lifshitz and Hancock 1981). The inoculum density (cfu = propagules) was determined by dilution plating on the *Pythium*-selective medium described by Mircetich and Kraft (1973). Inoculum of *R. solani* was prepared by the method of Ko and Hora (1971). The isolate was grown on moist autoclaved rye kernels (100 mL rye kernels + 150 mL water, soaked overnight, and autoclaved for 1 h on 2 consecutive days) for 2 weeks at 25°C, air dried in the dark for 24 h, ground, and sieved for particles of 0.6–1.5 mm diameter. Viability of the inoculum was tested by first weighing and then plating 200 particles on WA. The viability of propagules on WA was used to calculate the amount of inoculum required to adjust the inoculum density of *R. solani* in the soil.

Conidial suspensions of *F. solani* f.sp. *phaseoli* were prepared from 4-week-old cultures growing on potato dextrose agar (PDA) plates at 25°C under 12 h of fluorescent light (5000 lx) per day by scraping the culture surface with a glass rod after adding 10 mL of SDW/plate. The suspension was then homogenized in a Waring blender for 1 min and filtered through four layers of cheesecloth. The concentration of the conidial suspension was estimated with a haemocytometer.

Soil

A light sandy-loam soil, collected from a field near Georgetown, Ontario, which had no previous record of white bean cultivation, was used in all assays with *Pythium ultimum* and *R. solani*. Composite samples were collected from the upper 20 cm soil profile, bulked, and stored moist in covered containers at 16–18°C. For all experiments, soil was sieved (5-mm mesh) before use. Fine vermiculite (Plant Products Co. Ltd., Brampton, Ont.) was used for the assays with *F. solani* f.sp. *phaseoli*.

Effect of fungal inoculum density on disease severity

The effect of fungal inoculum density on disease severity for each pathogen on white bean seedlings was optimized prior to screening bacterial strains for biological control efficacy. The inoculum densities used in this study were based on results from preliminary tests to determine populations that consistently caused moderate amounts of disease.

Inoculum of *Pythium ultimum* was mixed thoroughly by hand into separate batches of soil to achieve an inoculum density of 0, 50, 100, and 200 propagules/g. Noninfested soil served as the control. The pathogen-amended and nonamended soil was then placed into 10 cm diameter plastic pots (300 g/pot). Four seeds of white bean cv. Seaforth were planted in each pot at a depth of approximately 2.5 cm. The following three treatments were used: (*i*) bean seeds planted in pathogen-free soil, (*ii*) bean seeds planted in pathogen-infested soil, and (*iii*) bean seeds planted in pathogen-infested soil that had been amended with the fungicide metalaxyl (Ciba-Geigy) to a final concentration of 10 μg of active ingredient (a.i.)/g of soil. The moisture content of the soil was maintained at 60%. Plants were fertilized once a week with 15-15-15 fertilizer suspension (NPK, 3 g/L). Experiments consisted of five replicate pots in each treatment. The pots were arranged in a completely randomized block design on a plant growth bench in a greenhouse maintained at 18–25°C, with 14 h of cool-white fluorescent light (10 000 lx) per day. After 4 weeks, all the surviving plants were gently pulled and the number of completely healthy plants devoid of any disease symptoms was determined. To confirm pathogen identity for seedlings with disease symptoms, infected tissues were plated onto *Pythium*-selective Mircetich medium (Mircetich and Kraft 1973).

Rhizoctonia solani inocula at 0, 1, 3, and 5 propagules/g soil were incorporated into soil and assays for disease development were conducted as previously described for *Pythium ultimum* except with the following modifications. Pentachloronitrobenzene (PCNB), at a rate of 20 μg a.i./g soil, was used instead of metalaxyl and seedlings with disease symptoms were plated onto 2% WA to confirm *R. solani* identity.

Conidia of *F. solani* f.sp. *phaseoli* at log 0, 3, 5, and 7 conidia/g fine vermiculite were tested in the same manner as for *Pythium ultimum*. In this case benomyl (benlate), at a rate of 20 μg a.i./g vermiculite, was used. Seedlings were removed and washed free of vermiculite 4 weeks after planting and disease severity was rated on a numerical scale of 0–5: 0 = healthy, symptomless plants; 1 = 1–10% root and (or) hypocotyls slightly discoloured ; 2 = 11–30% root rotted, damage with moderate discoloration of tissues; 3 = 31–50% root rotted with dark discolouration of tissues; 4 = 51–80% root rotted or completely collapsed; 5 = all of root rotted and (or) plant killed. Disease severity

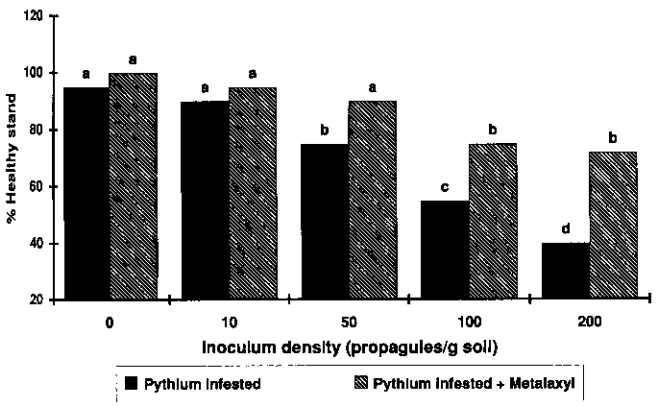

FIG. 1. Effect of *Pythium ultimum* inoculum density on the final healthy stand of white bean seedlings grown in soil for 4 weeks. Bars are means of two experiments with five replicated pots, each with four seedlings. Letters above the bars indicate significant difference: data within a treatment group that have the same letter do not differ significantly according to Fisher's protected LSD test ($P = 0.05$).

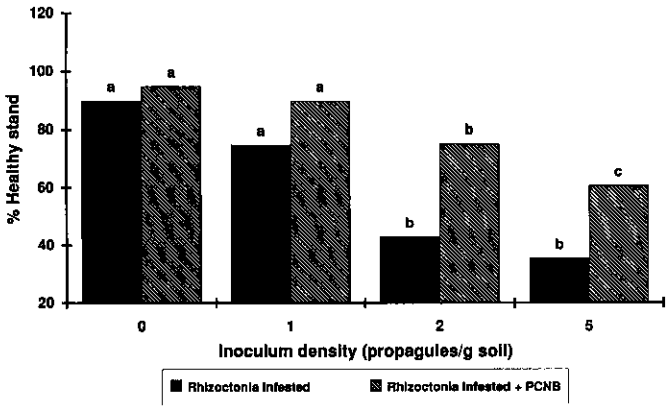

FIG. 2. Effect of *Rhizoctonia solani* inoculum density on the final healthy stand of white bean seedlings grown in soil for 4 weeks. Bars are means of two experiments with five replicated pots, each with four seedlings. Letters above the bars indicate significant difference: data within a treatment group that have the same letter do not differ significantly according to Fisher's protected LSD test ($P = 0.05$).

was calculated by averaging the infection rating of five replicates (pots). Tissues from infected roots were plated onto Nash-Snyder medium (Nash and Snyder 1962) supplemented with 5 mg/L benomyl (Hall 1981) to confirm *F. solani* f.sp. *phaseoli* as the infecting agent.

Screening bacteria for the suppression of preemergence damping-off and postemergence root rot of white bean seedlings

In several assays 120 bacterial strains (10 strains per assay for each pathogen at one time) were tested for their ability to protect white bean seedlings against each of the three fungal pathogens under the conditions previously described. Only one inoculum concentration of each fungus was tested on the basis of the results of the disease optimization assays. Inocula of *Pythium ultimum* and *R. solani* were tested at 200 and 3 propagules/g soil, respectively, and *F. solani* f.sp. *phaseoli* at log 3 conidia/g fine vermiculite.

Bacterial suspensions in SDW prepared as previously described were added to pathogen-amended sandy-loam soil or fine vermiculite at log 7 cfu/g soil. The soil or vermiculite was placed in autoclavable bags and mixed thoroughly by hand. The untreated control received only SDW without bacteria. The moisture content of the mixes was then adjusted to 60% with distilled water. The bags were sealed and incubated under greenhouse conditions for 1 week to allow the microbial populations to stabilize. Each assay consisted of seeds planted into (*i*) soil or vermiculite amended with a fungal pathogen, and (*ii*) soil or vermiculite amended with a pathogen and the test bacterium.

The experimental design and the conditions were as described previously. Postemergence root rot caused by *F. solani* f.sp. *phaseoli* and the number of healthy plants remaining in soils infested with *Pythium ultimum* and *R. solani* were recorded. Results were calculated as the percent difference compared with the pathogen controls.

In vitro antagonism on solid and liquid media between bacteria and fungi

The influence of each bacterial strain on the vegetative growth of *Pythium ultimum*, *R. solani*, and *F. solani* f.sp. *phaseoli* was examined on PDA (solid media). Plugs of mycelium (5 mm diameter) were cut from the edge of an actively growing fungal colony with a No. 2 cork borer, and one plug was placed in the centre of each PDA plate (100 × 15 mm). Two parallel 3.5 cm long streaks of bacteria were then made 2 cm apart on opposite sides of the plug. Plates were incubated in the dark for 3–7 days at 25°C after which time colony radius was measured and compared with that of colonies emerging from plugs not challenged by bacterial isolates. All tests with each bacterial isolate and pathogen combination were repeated three times.

The influence of each bacterial isolate on the growth of *Pythium ultimum*, *R. solani*, and *F. solani* f.sp. *phaseoli* in TSB (liquid media) was also assessed. A mycelium plug (5 mm diameter) from an actively growing fungal culture on PDA was placed in a Petri dish (100 × 15 mm) containing 10 mL of sterile TSB. The plate was then inoculated with 0.1 mL of a 24-h-old bacterial culture grown in TSB. Following incubation at 25°C in the dark for 7 days, the fungal mats were harvested by filtration with Whatman No. 1 filter paper. The mycelium was dried for 24 h at 65°C and the dry masses were determined. Mats from cultures not challenged by the bacterial isolates were used as controls. Each bacterial strain and fungal pathogen combination consisted of three replicates (plates) and the experiments were repeated twice.

Disease suppression was measured as the difference in percent healthy stand between the treatments with and without fungal pathogens. Percent healthy seedlings, radial growth, and mycelial dry mass differences compared with nontreated controls were calculated with the formula $(b - p)/p$, where b is the bacterial treatment and p is the control. Analysis of variance was performed on the data, using MSTAT-C (statistical software package from the Department of Crop and Soil Sciences, Michigan State University, East Lansing, Mich.). Treatment means were compared by calculating Fisher's protected least significance difference (LSD) at the $P = 0.05$ level of significance.

Results

Effect of fungal inoculum density on disease severity

Increasing levels of fungal inoculum density increased damping-off caused by *Pythium ultimum* (Fig. 1) and *R. solani* (Fig. 2) and root rot caused by *F. solani* f.sp. *phaseoli* (Fig. 3) on white bean seedlings. Two hundred propagules per gram of soil of *Pythium ultimum* and 3 propagules/g soil of *R. solani* and log 3 conidia/g fine vermiculite of *F. solani* f.sp. *phaseoli* provided a degree of disease severity on white bean seedlings selected here as optimal for testing the disease suppressiveness of the bacteria.

Influence of bacteria on suppression of preemergence damping-off and postemergence root rot of white bean seedlings

The bacterial strains introduced as soil treatments exhibited differential suppression effects on the damping-off caused by *Pythium ultimum* and *R. solani* and the root rot caused by *F. solani* f.sp. *phaseoli* on white bean seedlings. Although 120 strains were assayed for biocontrol efficacy, data from only those strains that showed clear suppression are included in this report, along with possible trends of the disease suppression. The identity and source of isolation of these strains are given in Table 1.

TABLE 1. Strains of bacteria used in this study

Strain		Source of isolation
1-102	*Serratia proteomaculans*	Soybean rhizosphere, Yellowknife, N.W.T.
1-133	*Serratia liquefaciens*	Corn rhizosphere, Yellowknife, N.W.T.
1-206	*Pseudomonas fluorescens*	Corn rhizosphere, Yellowknife, N.W.T.
17-34	*Pseudomonas fluorescens*	Soil, Alert, N.W.T.
2-20	*Serratia liquefaciens*	Soybean rhizosphere, James Bay
2-68	*Serratia liquefaciens*	Soybean rhizosphere, James Bay
25-33	*Pseudomonas putida*	Canola rhizosphere, Frobisher Bay, N.W.T.
33-2	*Pseudomonas putida*	Soybean rhizosphere, Sach's Harbour, N.W.T.
34-13	*Pseudomonas fluorescens*	Canola rhizosphere, Clyde, Alta.
55-14	*Pseudomonas putida*	Soil, Winnipeg, Man.
63-49	*Pseudomonas fluorescens*	Canola rhizosphere, Winnipeg, Man.
AC4-25	*Pseudomonas fluorescens*	Field soil, Georgia
AC4-39	*Pseudomonas* sp.	Field soil, Texas
AC4-40	*Enterobacter cloacae*	Field soil, Texas
AC4-75	*Pseudomonas cepacia*	Virginia Polytechnic Institute
AC4-105	*Pseudomonas fluorescens*	Soil, Labrador
AD4-34	*Pseudomonas cepacia*	Virginia Polytechnic Institute
G1-3	*Pseudomonas fluorescens*	Soil, Arctic
G1-5	*Flavobacterium* sp.	Soil, Arctic
G2-8	*Pseudomonas putida*	Thule peat, Resolute Bay, N.W.T.
G2-26	*Pseudomonas putida*	Thule peat, Resolute Bay, N.W.T.
G8-5	*Pseudomonas putida*	Grass rhizosphere, Craig Harbour
GR12-2	*Pseudomonas putida*	Soil, Arctic
L-25	*Pseudomonas fluorescens*	Cauliflower rhizosphere, Terra Nova, Labrador
L-26	*Pseudomonas fluorescens*	Cauliflower rhizosphere, Terra Nova, Labrador
MS-112	*Pseudomonas fluorescens*	Cotton rhizosphere, Mississippi
Ral-3	*Pseudomonas cepacia*	Soybean nodules, Alabama
RSC-6	*Pseudomonas fluorescens*	Soybean nodules, South Carolina
TR-21	*Pseudomonas fluorescens*	Cotton field soil, Mississippi
TR-24	*Pseudomonas* sp.	Cotton field soil, Mississippi
U-14	*Pseudomonas fluorescens*	Cotton field soil, Mississippi
U-15	*Pseudomonas* sp.	Cotton field soil, Mississippi

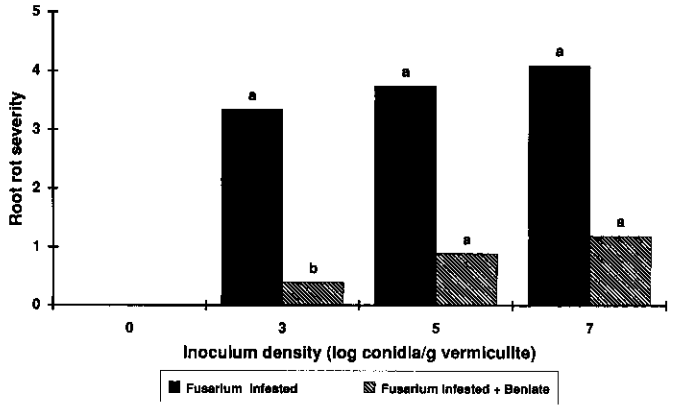

FIG. 3. Effect of *Fusarium solani* f.sp. *phaseoli* inoculum density on the severity of root rot of white bean seedlings grown in vermiculite for 4 weeks. Bars are means of two experiments with five replicated pots, each with four seedlings. Letters above the bars indicate significant difference: data within a treatment group that have the same letter do not differ significantly according to Fisher's protected LSD test ($P = 0.05$).

Two *Pseudomonas putida* strains (33-2 and G8-5), six *Pseudomonas fluorescens* strains (AC4-25, AC4-105, U-14, 34-13, TR-21, and L-25), and one *Pseudomonas* sp. (AC4-39) significantly ($P = 0.05$) suppressed damping-off disease caused by *Pythium ultimum* (Table 2).

Significant ($P = 0.05$) suppression of damping-off caused by *R. solani* was only observed with four strains of *Pseudomonas fluorescens* (MS-112, U-14, 17-34, and L-26) and one *Pseudomonas* sp. (U-15) among 120 strains tested (Table 3).

Nine bacterial strains belonging to *Pseudomonas fluorescens* (L-26, RSC-6, 63-49, 34-13, and U-14), one *Pseudomonas cepacia* (Ral-3), two *Pseudomonas putida* (G2-26 and G2-8), and one *Pseudomonas* sp. (TR-24) significantly ($P = 0.05$) reduced root rot severity caused by *F. solani* as compared with the bacteria-free controls (Table 4).

Only strains of *Pseudomonas fluorescens* were effective against more than one pathogen. Strain U-14 suppressed all three pathogens (Tables 2–4). Strain 34-13 gave significant protection against *F. solani* f.sp. *phaseoli* and *Pythium ultimum*, and strain L-26 significantly suppressed *F. solani* f.sp. *phaseoli* and *R. solani*.

In vitro antagonism

Three strains (AC4-105, AC4-40, and G1-3) significantly ($P = 0.05$) inhibited the radial growth of *Pythium ultimum* on solid media and 11 strains significantly ($P = 0.05$) reduced the growth of mycelia of *Pythium ultimum* in liquid media when compared with the controls (Table 2). There was a positive relationship for only four strains (U-14, AC4-39, 34-13, and 33-2) between solid and liquid media on vegetative growth inhibition of *Pythium ultimum* (Table 2).

TABLE 2. Relative efficacy of bacteria between in vitro inhibition of vegetative growth and greenhouse suppression of damping-off of *Pythium ultimum* on white bean seedlings

Strain	Suppression of damping-off (% difference in healthy seedlings relative to pathogen control)	Antibiosis on solid media (% difference in radial growth relative to nonbacterial control)	Antibiosis in liquid media (% difference in mycelial dry mass relative to nonbacterial control)
33-2	25.0*	−6.1	−91.8*
RSC-6	18.8	−28.1	−94.0*
2-68	18.8	−19.9	−89.9*
25-33	18.8	−23.4	−84.9*
G2-26	−10.5	0	0
G8-5	25.0*	0	0
AC4-25	350.0*	−17.7	−26.9
AC4-105	225.0*	−32.0*	−61.9*
U-14	200.0*	−21.5	−85.4*
AC4-39	150.0*	−13.6	−70.0*
34-13	26.7*	−2.0	−90.3*
TR-21	26.7*	1.4	0
L-25	26.7*	0	−32.9
1-102	13.3	−24.4	−93.5*
1-206	12.5	−32.0	−84.8*
AC4-40	−10.5	−35.7*	−35.1*
G1-3	21.4	−57.9*	nt

NOTE: Data for suppression of damping-off are means calculated from five replications per treatment with four seedlings per replication. Data for antibiosis on solid and in liquid media are means of three replications per treatment. Percent difference of healthy seedlings, radial growth, and mycelial dry mass relative to nontreated controls were calculated with the formula $(b - p)/p$, where b is the bacterial treatment and p is the control. See Table 1 for strain identification and source of isolation. nt, not tested.

*Significantly different according to Fisher's protected LSD at P = 0.05 from nontreated controls.

TABLE 3. Relative efficacy of bacteria between in vitro inhibition of vegetative growth and greenhouse suppression of damping-off of *Rhizoctonia solani* on white bean seedlings

Strain	Suppression of damping-off (% difference in healthy seedlings relative to pathogen control)	Antibiosis on solid media (% difference in radial growth relative to nonbacterial control)	Antibiosis in liquid media (% difference in mycelial dry mass relative to nonbacterial control)
AC4-75	23.1	−2.4	−99.0*
2-68	0	−9.9	−100.0*
25-33	−40.0	−9.2	−54.9*
55-14	−20.0	−5.3	−94.2*
MS-112	38.5*	−1.4	−39.8
1-133	−16.7	−17.2	−99.9*
AD4-34	−6.3	−1.9	−92.8*
U-14	38.5*	−5.7	−100.0*
GR12-2	18.8	0	−65.0*
RSC-6	13.0	−54.2*	−63.1*
G1-3	32.5	−33.5*	−99.3*
AC4-40	11.1	−22.5	−85.9*
63-49	5.0	−39.3*	−84.5*
17-34	100.0*	−12.8	−98.6*
U-15	33.5*	−18.1	−99.6*
L-26	33.3*	−0.4	−34.3

NOTE: See Table 2 for explanations.

Three bacterial strains (RSC-6, G1-3, and 63-49) significantly ($P = 0.05$) inhibited the vegetative growth of *R. solani* on solid media and 14 strains reduced the growth of mycelia in liquid media compared with controls (Table 3). A positive relationship was found for only two strains (RSC-6 and 63-49) on significant ($P = 0.05$) inhibition of the growth of *R. solani* on solid and liquid media. There were seven strains on solid media and another seven in liquid media that significantly ($P = 0.05$) inhibited the growth of *F. solani* f.sp. *phaseoli* (Table 4). Four strains (63-49, 2-20, AC4-75, TR-24, and 34-13) significantly ($P = 0.05$) inhibited the vegetative growth of *F. solani* f.sp. *phaseoli* on both solid and liquid media.

None of the rhizobacteria tested were capable of inhibiting the growth of all three fungal pathogens on solid media (Tables 2–4 and data not presented). Only one strain (U-14) significantly inhibited the growth of *Pythium ultimum*, *R. solani*, and *F. solani* f.sp. *phaseoli* in liquid media. Strain G1-3 significantly inhibited the growth of *Pythium ultimum* and *R. solani*, and strains RSC-6 and 63-49 significantly inhibited the growth of *R. solani* and *F. solani* f.sp. *phaseoli* on solid media. Also strain 63-49 on *R. solani* and *F. solani* f.sp. *phaseoli*, and strains 2-68 and 25-33 on *Pythium ultimum* and *R. solani* were significantly effective in reducing the mycelial growth in liquid media.

TABLE 4. Relative efficacy of bacteria between in vitro inhibition of vegetative growth and greenhouse suppression of postemergence root rot of *Fusarium solani* f.sp. *phaseoli* on white bean seedlings

Strain	Suppression of root rot (% difference in root rot relative to pathogen control)	Antibiosis on solid media (% difference in radial growth relative to nonbacterial control)	Antibiosis in liquid media (% difference in mycelial dry mass relative to nonbacterial control)
L-26	92.7*	−9.3	−15.6
RSC-6	52.4*	−54.6*	16.5
2-20	5.0	−31.9*	−55.0*
AC4-75	10.4	−40.1*	−58.3*
RAL-3	60.6*	−56.3*	−12.8
AC4-105	6.5	−26.8	−26.1
G1-3	27.0	−20.0	nt
TR-24	95.1*	−36.9*	−70.0*
G2-8	78.5*	8.9	−50.6*
63-49	53.3*	−42.9*	−17.2
G2-26	52.1*	6.5	−31.1*
34-13	50.2*	−40.1*	−47.8*
U-14	47.1*	−28.2	−43.3*
G1-5	nt	−7.8	nt
AC4-40	27.6	−9.1	nt

NOTE: See Table 2 for explanations.

Discussion

Selection of effective bacterial strains prior to testing under commercial conditions is the major bottleneck in the development of useful biocontrol agents. The goal of the assays described in this study was to improve the chances of selecting good candidate strains that can suppress soil-borne root pathogens in the field. A preliminary test to identify competent strains that inhibit diseases caused by *Pythium ultimum*, *R. solani*, and *F. solani* f.sp. *phaseoli* would greatly accelerate the selection process. The bacterial strains used in this study, initially selected for their ability to colonize roots of agricultural crops, were in most cases not highly effective as suppressants of *Pythium ultimum*, *R. solani*, and *F. solani* f.sp. *phaseoli* on white bean seedlings (Tables 2–4). Out of the 120 bacterial strains tested only 7.5, 4.2, and 7.5% were effective in suppressing diseases on white bean seedlings caused by *Pythium ultimum*, *R. solani*, and *F. solani* f.sp. *phaseoli*, respectively, when introduced to the growing mix.

In vitro antibiosis of bacteria against several soil-borne pathogenic fungi has been demonstrated numerous times. However, only a few reports relate such activities to antagonism in vivo (Howell and Stipanovic 1980). Our study does not indicate any correlation between antibiosis on solid or liquid culture media and suppression of disease on plants grown in a potting mix. This is not unexpected, as numerous mechanisms are considered to be involved in biological control, including competition for nutrients and siderophore production, to name just two.

Inhibitory substances thus may not always be the fundamental requirements for biological control activity. Indirect effects such as hyphal agglutination and bacterial enhancement of host resistance to plant pathogens may also be important mechanisms. Therefore, selection of biocontrol agents solely on the basis of an in vitro antibiosis assay risks loss of beneficial organisms that provide protection by other mechanisms. Exploitation of microbial treatments may have to await a greater understanding of soil microbiology and the underlying mechanisms involved in biological control.

Although many questions remain to be addressed regarding the bacterial strains reported herein, some of the strains tested have potential as biocontrol agents for preemergence damping-off in white bean seedlings and postemergence root rot control. Field trials with these strains are being conducted.

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